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Detection of linked QTL for soybean brown stem rot resistance in 'BSR 101' as expressed in a growth chamber environment*

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Abstract

The objective of this study was to map the gene(s) conferring resistance to brown stem rot in the soybean cultivar BSR 101. A population of 320 recombinant inbred lines (RIL) was derived from a cross of BSR 101 and PI 437.654. Seedlings of each RIL and parent were inoculated by injecting stems with a suspension of spores and mycelia of *Phialophora gregata*, incubated in a growth chamber at 17 °C, and assessed for resistance by monitoring the development of foliar and stem symptoms. The population also was evaluated with 146 RFLPs, 760 AFLPs, and 4 probes for resistance gene analogs (RGAs). Regression analysis identified a significant association between resistance and several markers on Linkage Group J of the USDA-ARS molecular marker linkage map. Interval analysis with Mapmaker QTL identified a major peak between marker RGA2V-1 and AFLP marker AAGATG152M on Linkage Group J. A second peak, associated only with stem symptoms, was identified between the RFLP B122I-1 and RGA2V-1, also on Linkage Group J. When composite interval mapping with QTL Cartographer was used, two linked QTL were identified with both foliar and stem disease assessment methods: a major QTL between AFLP markers AAGATG152E and ACAAGT260, and a minor QTL between RGA3I-3 and RGA3I-2. These results demonstrate that composite interval mapping gives increased precision over interval mapping and is capable of distinguishing two linked QTL. The minor QTL associated with the cluster of RGA3I loci is of special interest because it is the first example of a disease resistance QTL associated with a resistance gene analog.

Introduction

Brown stem rot (BSR) is one of the most frequently occurring and devastating diseases of soybean (*Glycine max* (L.) Merr.) in the North Central U.S. and in Canada [2]. It is caused by the soil-borne fungus *Phialophora gregata* (Allington and Chamberlain) W. Gams. Symptoms include interveinal chlorosis, necrosis, premature leaf abscission, internal stem browning, and grain yield loss. Infected plants can exhibit reduced photosynthate availability during seed filling that results in yield loss as high as 66% [9].

Resistance to BSR was first identified in PI 84946-2 [5]. Resistance is controlled by two dominant genes in PI 84946-2 and by a single dominant gene in L78-4094, derived from PI 84946-2 [27]. The single resistance gene in L78-4094 was designated Rbs_1. Allelism tests with L78-4094 and PI 437833 were used to distinguish Rbs_1 from a second resistance gene, designated Rbs_2 [14]. A third resistance gene, Rbs_3, was identified in PI 437970 [38]. Other resistance sources have been identified, but their genetic control has not yet been determined [24, 36, 37, 39].

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Traditional breeding methods have been used to develop resistant cultivars with high grain yield and other good agronomic traits. However, in environments with low levels of *P. gregata*, grain yield of most resistant cultivars has been less than that of susceptible cultivars [30]. Sebastian *et al.* [30] suggest that it should be possible to develop resistant lines that yield well regardless of pathogen level, but traditional breeding methods have been slow to reach this goal.

The primary obstacle to development of high-yielding resistant lines is the low heritability of resistance. A wide range of broad-sense heritability estimates, ranging from 0.21 to 0.88, was reported by Sebastian *et al.* [29] for glasshouse-grown F_2 populations. In 12 years of evaluation of PI 84946-2, the source of resistance for all publicly released resistant cultivars, disease incidence ranged from 5 to 64% [5] on heavily infested soils. All of the studies on inheritance of resistance have reported high χ^2 values due to skewed ratios of progeny from crosses of resistant and susceptible lines. The direction of skewedness was not consistent among studies, indicating a large environmental effect on expression of resistance. Low heritability can be the result of multiple genes controlling the trait, a large environmental effect, or a combination of both factors. Genetic tests with the public cultivar BSR 101, derived from PI 84946-2, indicated that resistance is determined by a single resistance gene allelic to *Rbs3* and possibly an additional modifier gene [10]. However, the results also could have been interpreted to show that resistance is controlled by a single gene that is strongly affected by environment.

For example, incidence of BSR has been associated with incidence of other soybean diseases. Sugawara *et al.* [33] found that infection by soybean cyst nematode (*Heterodera glycines*) increases the severity of BSR in cultivars susceptible to BSR. G.L. Tylka and J.E. Behm (personal communication) also have observed this effect in both susceptible and resistant cultivars, including BSR 101. Also, Tachibana and Card [34] reported that soybean mosaic virus incidence decreased expression of BSR. However, Lohnes and Nickell [21] reported a positive association between soybean mosaic virus and powdery mildew incidence. Interactions among these diseases are not understood, and reflect some of the complexity of BSR expression.

Several methods have been used to evaluate BSR resistance. Early studies of inheritance of BSR resistance were conducted in naturally infested fields [5]. To reduce environmental effects, more recent genetic studies have been conducted in glasshouses. Sebastian *et al.* [28] assessed BSR resistance by inoculating plants grown in a glasshouse maintained at 20 °C. The predominant method of inoculation in glasshouse environments has been by root-dip, that is, uprooting the plants and immersing the roots in a suspension of *P. gregata* mycelial fragments and conidia prior to replanting [10, 14, 36, 38]. Some investigators have used stem browning [12, 35], and others have used leaf chlorosis and necrosis as a measure of relative disease resistance [28, 36]. Sebastian *et al.* [29] reported that measurement of leaf symptoms resulted in higher heritability estimates than measurement of stem symptoms in glasshouse environments and was, therefore, more useful for assessing resistance in soybean lines.

Because of the low heritability of BSR resistance, genetic studies and development of high-yielding resistant cultivars would be greatly facilitated by marker assisted selection. The objective of this study was to map the gene or genes conferring resistance to BSR in BSR 101, thereby identifying several molecular markers for use in marker assisted selection.

Materials and methods

Soybean population

A population of 320 recombinant inbred lines (RIL) in the F6:7 generation was derived from a cross of BSR 101, a BSR-resistant line with good agronomic traits, and PI 437.654, a BSR-susceptible line [3] which is of agronomic importance because of its resistance to all known races of soybean cyst nematode in the USA [8, 23].

Disease resistance evaluation

Soybeans were planted in a mix of soil, sand, and perlite (2:1:2) in 4 × 21 cm plastic cone-shaped containers (Stuewe & Sons, Corvallis, OR). The seedlings were maintained in a glasshouse at 27 °C with 14 h/day supplemental light from sodium discharge lamps. After the appearance of unifoliate leaves, seedlings were fertilized weekly with a 20-20-20 fertilizer solution (Robert B. Peters Co., Allentown, PA). Vigorous seedlings were inoculated at growth stage V1-V2 [11], usually 19 days after planting.

Phialophora gregata isolate OH2-3, derived from a single spore of isolate OH2 [10], was used for all inoculations. The OH2-3 stock was stored as conidia

and mycelial fragments in 15% glycerol at −75 °C. Inoculum was prepared by growing the fungus on green bean extract agar (30 g/l frozen cut green beans, 20 g/l agar) for five weeks at 24 °C with ambient light. Spores were harvested by washing the agar surface with sterile demineralized water and lightly scraping the surface of the agar with a spatula. Spore concentration was determined using a hemacytometer. Spores were resuspended to a concentration of 10^8 spores/ml in sterile 0.7% water agar. The spore suspension was stored on ice until used for plant inoculations.

Seedlings were inoculated 1 cm above the soil line by scooping the inoculum suspension (about 1.4 μl containing about 1.4×10^5 spores) with the bevel of a sterile 22 gauge hypodermic needle and stabbing the stem through the stele. To minimize environmental effects and enhance symptom development, inoculated seedlings were maintained in growth chambers at 17 °C [1] with 14 h light per day at 530 μE $cm^{-2}s^{-1}$. Controls were parental lines stabbed with a sterile needle.

Disease severity was estimated by measuring the proportion of necrotic or abscised foliar tissue and by measuring the percentage of discolored stem tissue. Foliar symptoms were recorded at 3.5, 4.0, and 4.5 weeks after inoculation. Each leaflet was recorded as healthy, chlorotic, or necrotic depending on the predominant (>50%) condition of the leaflet. Abscised leaflets were recorded as missing. Foliar disease severities were calculated using the following formula: (necrotic leaflets + missing leaflets)/total leaflets. Foliar disease severities ranged from 0% (healthy) to 100% (all leaflets dead or missing). In these environmental conditions, chlorosis was poorly correlated with disease development; even uninoculated plants tended to become chlorotic. Therefore, chlorotic leaflets were not included in the calculations for foliar disease severity. Stem browning was measured one day after the 4.5-week foliar symptoms were recorded. Stem symptoms were recorded by measuring plant height above the wound, then splitting the plant lengthwise and measuring from the wound to the highest point of browning. The browning in the affected area was compared to a standard area diagram (Figure 1) to estimate the percentage of discolored tissue. Total severity of stem browning was calculated using the following formula: (highest point of browning/plant height) × percent discoloration. Stem browning ranged from 0% (healthy) to 60% (extreme browning).

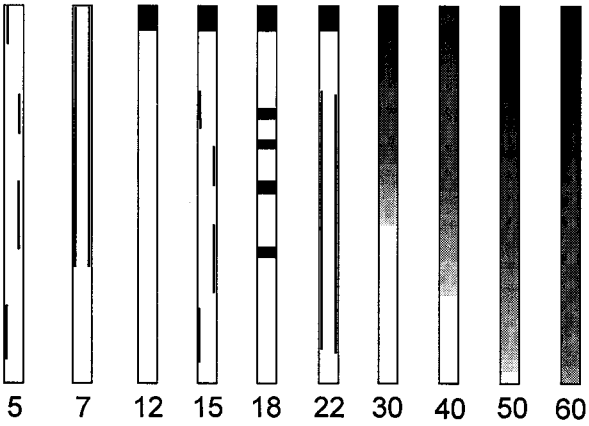

Figure 1. Standard area diagram used to determine the percentage browning in stems of BSR-infected soybeans. The numbers indicate the proportion of area discolored (%).

Three seedlings of each RIL and 84 seedlings of each parent were inoculated and placed in each of two growth chambers. An analysis of variance was conducted on the foliar and stem disease ratings. The formula, $H^2 = \sigma_G^2/((\sigma_E^2/r) + \sigma_G^2)$, where r is number of replications, was used to calculate broad-sense heritability.

Molecular marker evaluation and mapping

DNA from the parents and the 320 RILs was isolated from a bulk of young first-trifoliolate leaves of at least 30 seedlings per line. The DNA extraction, blotting, hybridization, and autoradiography methods used followed Keim *et al.* [17]. For each marker, observed phenotypic segregation ratios were compared with the expected 1:1 ratio of parental phenotypes by χ^2 analysis, and any marker that did not fit the expected ratio ($P < 0.05$) was excluded from further analyses. AFLP marker data were obtained as stated in Kiem *et al.* [16].

In order to quickly make a preliminary molecular map and determine which linkage groups were associated with resistance, two subsets of the RIL population were analyzed initially. One subset of 100 RILs was genotyped with 267 RFLP probes [18], and another subset of 42 RILs was genotyped using 760 AFLP markers [16]. Prior to identification of markers associated with resistance, separate molecular marker maps were made using the RFLP and AFLP data subsets. After the identification of markers associated with resistance, the remaining RILs were analyzed for AFLP (278 RILs) and RFLP (220 RILs) markers associated with resistance in order to make a single more precise

map. Anchor RFLP loci, utilizing identical probe-enzyme combinations and banding patterns, were used to identify linkage groups defined by the USDA-ARS public map [32]. Additional molecular markers expected to be linked to those found associated with BSR resistance were used to genotype the 320 RILs. These additional markers included probes produced by polymerase chain reaction (PCR) using primers designed from sequences of four RGAs [15].

All molecular marker maps were constructed using Mapmaker 3.0 [20] (MS-DOS version). The RIL (ri-self) genetic model, minimum LOD score of 3.0, Haldane estimation [13], and maximum recombination of 30% were used. The 'compare', 'try', and 'ripple 6' (minimum LOD score of 3.0) commands were used to assign gene orders.

Trait association with molecular markers

Three methods were used to detect quantitative trait loci (QTL). The first was SAS [26] regression analysis because of its ability to detect marker association with a trait regardless of whether the marker is linked to other markers. The second was interval mapping using Mapmaker QTL [19] and QTL Cartographer, Model 3 [4]. Unlike QTL Cartographer, Mapmaker QTL was not designed for analysis of recombinant inbred line data, but, because of a perceived lack of any other software to analyze these populations, previously has been used for such analysis following initial regression analysis identification of QTL. The third QTL detection method used was composite interval mapping using QTL Cartographer, Model 6, [4] because of its ability to detect linked QTL. Each analysis was conducted on all three data sets: the mean for each line within each of the two growth chambers; and the mean for each line across both growth chambers.

Regression analysis of trait and marker data was conducted using the PROC GLM procedure of SAS [26]. The probability of association of each trait with each marker was determined to identify QTL. To minimize detection of false associations, a significant association was declared only if the probability of a greater F value was less than or equal to 0.005. The proportion of genotypic variation explained by the marker with the greatest R^2 value was estimated by dividing the R^2 value for that marker by the trait heritability [7].

RFLP and AFLP markers associated with BSR resistance were analyzed next with the interval mapping software Mapmaker QTL [19]. To adapt Mapmaker QTL for use with an RIL population, the 'data type' statement was changed from 'ri self' to 'f2 intercross'; the data were not changed. This analysis gives inaccurate recombination estimates for an RIL population but can identify marker intervals more likely to contain a QTL.

QTL Cartographer [4] also was used to identify and localize QTL. Output files from Mapmaker were used as input files for QTL Cartographer sub-program Rmap. The sub-program Qstats was used to create trait distribution histograms. The sub-programs Lrmapqtl and Zmapqtl were used to identify markers associated with BSR resistance. Zmapqtl identifies intervals likely to contain QTL and, when Model 3 is used in the analysis, the output information is similar to that of Mapmaker QTL (interval mapping) except that, for RIL as well as F_2 populations, the recombination rates given in the output are in agreement with Mapmaker output. When Model 6 is used in the analysis (composite interval mapping), Zmapqtl adjusts the analysis to control the effects of genetic background and increases the precision of the analysis. The suggested five background markers and 10 cM window size was used in the first analysis. To increase precision, the number of background markers was increased to 20, one less than the total number of markers on our Linkage Group J, and the window size was decreased to 2 cM.

Results

Disease resistance evaluation

Histograms showing the frequency distribution of soybean RILs with various levels of BSR resistance are given in Figure 2. The average foliar disease severity rating for inoculated plants in both growth chambers and for all symptom measurement dates for PI 437.654 was 77%, and for BSR 101 was 5%; the least significant difference value (LSD) ($P < 0.05$) was 35%. The average stem severity for PI 437.654 was 51%, and for BSR 101 was 2%; the LSD ($P < 0.05$) was 22%. Foliar and stem disease severity ratings for wounded but uninoculated plants were 0%. No RIL had foliar or stem symptoms significantly more severe than did PI 437.654 or less severe than BSR 101. The absence of transgressive segregant lines suggests that, if multiple BSR resistance genes are segregating in this population, the resistance alleles likely are from BSR 101 and not PI 437.654.

The broad-sense heritability calculated from foliar disease ratings was 0.66, and from stem ratings 0.73.

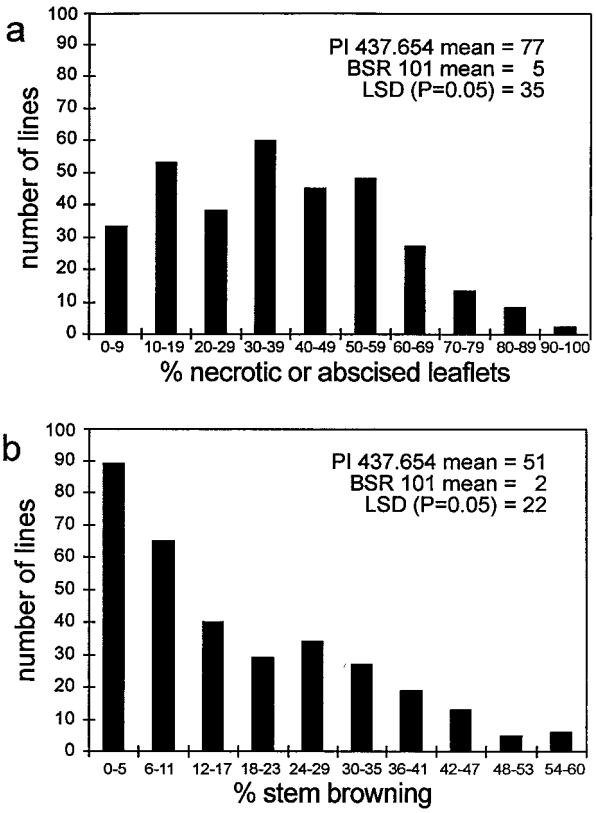

Figure 2. Numbers of soybean recombinant inbred lines from a cross of BSR 101 and PI 437.654 versus the levels of brown stem rot resistance as measured by foliar and stem ratings. Foliar (a) and stem (b) disease severity ratings for each line were averaged from measurements taken on three seedlings in each of two growth chambers.

These heritability values are within the range of those reported by Sebastian *et al.* [29] for glasshouse-grown material. Correlation of leaf and stem symptom data from both growth chambers was 0.72 (Figure 3). The high correlation and the similar heritabilities suggest that either foliar or stem symptoms can be used to evaluate growth-chamber grown breeding lines from a cultivar development program. An obvious advantage of using foliar symptoms is that it is non-destructive and does not require the manual effort of cutting open thin stems. Foliar symptoms, therefore, can be measured at intervals, but the stem symptoms can be measured only once. This advantage, combined with the observation that even the resistant parent, BSR 101, eventually succumbed to the disease after several weeks, suggests that use of foliar symptoms may be more prudent.

Differences in disease severity ratings between the growth chambers were significant when either foliar

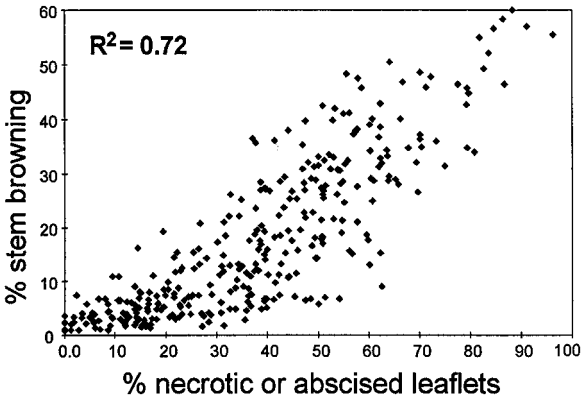

Figure 3. Average percentage of stem browning plotted against average foliar disease severity for each soybean recombinant inbred line derived from the cross of BSR 101 and PI 437.654. Values for each line were averaged from measurements taken on three inoculated seedlings in each of two growth chambers.

or stem symptoms were recorded. The average foliar disease severity was 47% for inoculated plants in growth chamber 1, and 28% for those in growth chamber 2. The average stem browning was 23% for inoculated plants in growth chamber 1, and 13% for growth chamber 2. The differences between growth chambers support earlier observations that expression of BSR resistance is strongly affected by factors other than genotype and confirms the importance of marker development for use in selection.

Genetic map and trait association

A total of 146 RFLP markers and 760 AFLP markers was used to develop genetic linkage maps for this population; 27 linkage groups were defined with the RFLP markers, while 20 markers remain unlinked; 28 linkage groups were defined by the AFLP markers [16].

A single linkage group, Linkage Group J of the USDA-ARS molecular marker linkage map [32], was identified by all three QTL detection methods used, SAS regression analysis, interval mapping using Mapmaker QTL, and QTL Cartographer, Model 3, as being associated with BSR resistance (Figure 4a, b). Although differences between growth chambers were observed for the mean disease severity ratings of lines, QTL analysis of data from each growth chamber and across the two growth chambers were in agreement.

No evidence was found for QTL other than on Linkage Group J. This possibility was tested because Eathington *et al.* [10] had suggested, based on skewed ratios of progeny types and high χ^2 values in allelism

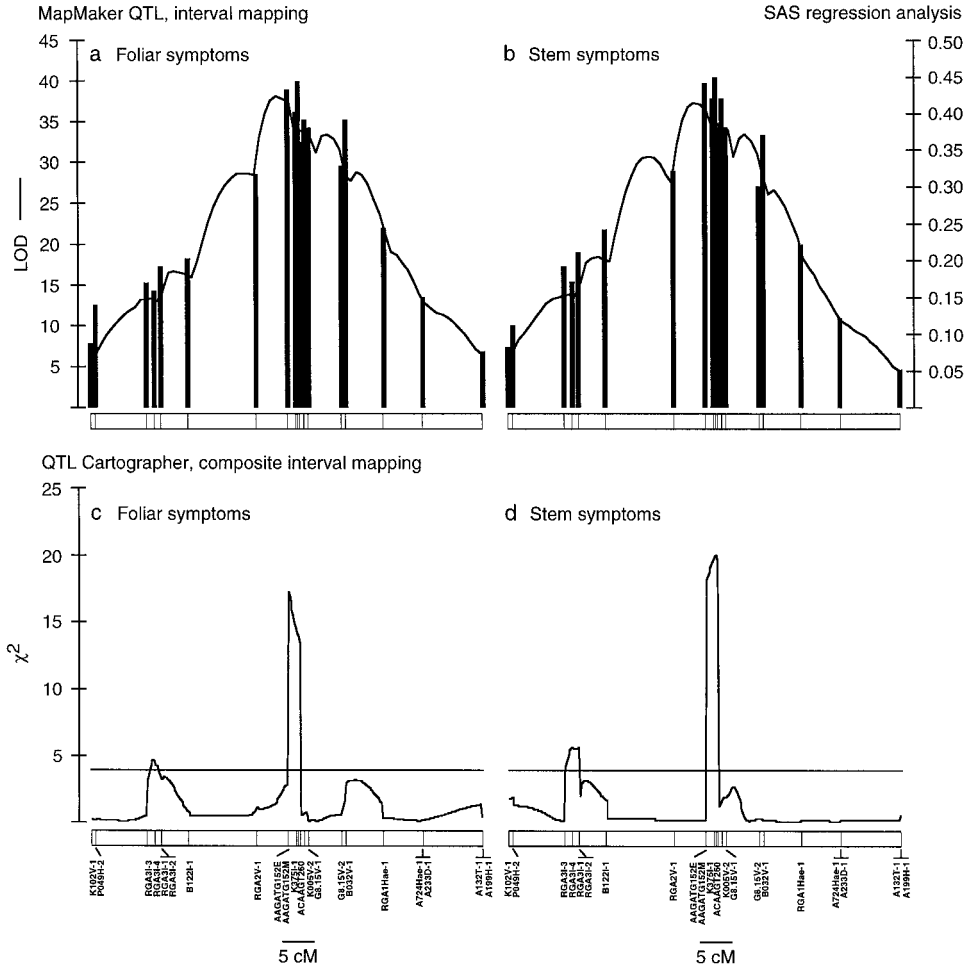

Figure 4. Soybean Linkage Group J from the BSR 101 by PI 437.654 recombinant inbred line population, showing marker associations with brown stem rot resistance as measured by foliar disease severity (A and C) and stem browning (B and D). Associations are illustrated in A and B by vertical bars for R^2 values from SAS linear regression analysis, and plot of LOD scores from Mapmaker QTL. Associations are illustrated in C and D by a curve from QTL Cartographer. The horizontal bar indicates significance at $P < 0.05$.

tests, that resistance was controlled by two genes, one with a major effect, and a second with a minor effect. They also suggested that the second locus might be linked to the first. In an attempt to identify additional QTL, the data set was divided by RIL according to the molecular marker alleles at the loci most closely associated with resistance. This resulted in two subsets of RILs. One subset had BSR 101 alleles, and the other set had PI 437.654 alleles at the loci most closely associated with resistance. Within each of these subsets, any variability in genetically attributed BSR expression was expected to be due to QTL in regions of the genome other than the first region identified. Each subset was then reanalyzed with SAS linear regression to identify any additional significant QTL. Regression analysis of the two data sets divided according to alleles at marker loci most correlated with resistance did not identify any additional QTL. This analysis could not eliminate the possibility that the QTL on Linkage Group J could be the result of two or more closely linked resistance genes, and it is not uncommon for DNA sequences to be duplicated and clustered within a linkage group [31].

Using regression analysis of markers and disease severity to detect QTL, all of Linkage Group J, from RFLP markers K102V-1 through A199H-1, was significantly correlated with BSR resistance by regression analysis of markers and disease severity. The maximum association was observed at the cluster of markers from AFLP marker AAGATG152E to RFLP

marker G815V-1. R^2 values ranged from of 0.32 to 0.40 for foliar disease severity (Figure 4a) and 0.38 to 0.45 for stem browning (Figure 4b). The marker with the greatest association with resistance was K375I-1. The proportion of genotypic variation explained by K375I-1 (R2/H) was 0.61 when foliar symptoms were used to assess disease severity, and 0.62 when stem symptoms were used.

To more precisely locate QTL, interval mapping using Mapmaker QTL [19] and QTL Cartographer, Model 3 [4], was used. Mapmaker QTL analysis of foliar disease ratings identified a single LOD peak of 38.04 between RGA2V-1 and AFLP marker AAGATG152M (Figure 4a). When stem browning was used as a measure of disease severity, two peaks were identified: one with a LOD of 41.20 between RGA2V-1 and AAGATG152M, and the second with a LOD of 33.74 between B122I-1 and RGA2V-1 (Figure 4b). Interval mapping analysis using QTL Cartographer, Model 3, gave results similar to those of Mapmaker QTL.

Identification of linked QTL

Composite interval mapping of foliar disease severity ratings using QTL Cartographer with Model 6 identified a major QTL with a peak χ^2 value of 17.37 between AFLP markers AAGATG152E and ACAAGT260 (Figure 4c) along with a second QTL with a peak χ^2 value of 4.64 between markers RGA3I-3 and RGA3I-2. Analysis of stem browning symptoms identified QTL in the same intervals; the major QTL had a peak χ^2 value of 20.2, and the minor QTL had a peak χ^2 value of 5.58 (Figure 4d). Resistance was associated with the BSR 101 allele at both loci. The major and minor QTL were identified using the analysis of data from each growth chamber separately and also the average of data across growth chambers. These results demonstrate that composite interval mapping using QTL Cartographer is capable of distinguishing two linked QTL.

To determine the significance of the effect of an allele difference at the markers most closely associated with each of the two QTL, LSD values were calculated for comparison of average disease ratings of genotypes with all possible allele combinations at the two QTL (Table 1). The effect of an allele difference at the major QTL was significant regardless of whether the resistant or susceptible allele was present at the minor QTL. The effect of an allele difference at the minor QTL was significant only if the allele present at the major QTL was from the susceptible parent. No epistatic effects were detected.

Discussion

QTL analysis of foliar and stem symptom data collected from plants inoculated in a controlled-environment chamber has identified one major and one minor QTL in Linkage Group J responsible for disease resistance in BSR101. This result is consistent with the suggestion of Eathington *et al.* [10] that resistance in BSR101 is controlled by two genes, one with a major effect (*Rbs3*), and a second with a minor effect. Eathington's suggestion was based on skewed ratios of progeny types and high χ^2 values in allelism tests using glasshouse-grown plants. He also suggested that the second locus might be linked to the first. Our results also are consistent with a QTL analysis of disease symptom data collected in field environments that identified an association of BSR resistance with markers only on Linkage Group J (D.M. Webb. 1987. Brown stem rot resistance in soy beans, U.S. Patent 5,689,035. Date issues: 18 November). However, unlike the field studies, we were able to detect the presence of a linked, minor QTL. The greater definitiveness of our studies may be attributable to more consistent disease development in the growth chamber environment, and the increased precision of analysis with composite interval mapping (Figure 4).

The mapping of BSR resistance to Linkage Group J is especially interesting because this linkage group is already known to contain several disease resistance genes. Polzin *et al.* [25] mapped the powdery mildew (*Microsphaera diffusa* Cooke & Peck) resistance locus, *Rmd*, the *Phytophthora* root and stem rot (*Phytophthora sojae* Kaufmann & Gerdemann) resistance locus, *Rps2*, and the *Rj2* locus, controlling ineffective nodule formation by *Bradyrhizobium japonicum* (Kirchner) Jordan, to Linkage Group J between RFLP loci A233D and A199H. A minor QTL for resistance to soybean cyst nematode (*Heterodera glycines*) also is associated with marker B032V on Linkage Group J [6]. In addition, several resistance gene analogs (RGA1, RGA2, RGA3, RGA5, and RGA6) have been mapped to Linkage Group J [15].

The presence of genes conferring resistance to BSR on the same linkage group as a variety of other disease resistance genes may explain some of the previously reported correlations between BSR resistance and resistance to other diseases. For example, linkage

Table 1. Average brown stem rot disease ratings, and the differences between disease ratings (LSD $P = 0.05$) for groups of soybean recombinant inbred lines from a cross of BSR 101 and PI 437.654. Alleles from BSR 101 (B) are associated with resistance, and alleles from PI 437.654 (P) are associated with susceptibility at two resistance QTL on Linkage Group J. A major QTL is associated with RFLP marker K375I-1 (QTL1), and a minor QTL is associated with PCR-product marker RGA3I-4 (QTL2).

Disease rating	Genotype and number (N) of RIL within genotypic class		Difference
	$QTL1_B$ $QTL2_B$ ($N = 95$)	$QTL1_B$ $QTL2_P$ ($N = 40$)	
Foliar rating	2.2	2.8	0.4 (LSD=0.53)
Stem rating	7.2	8.6	1.4 (LSD=2.23)
	$QTL1_P$ $QTL2_B$ ($N = 41$)	$QTL1_P$ $QTL2_P$ ($N = 108$)	
Foliar rating	4.9	5.4	0.5 (LSD=0.49)
Stem rating	21.1	28.4	7.3 (LSD=3.65)
Difference between foliar ratings	2.5 (LSD=0.49)	2.6 (LSD=0.54)	
Difference between stem ratings	13.9 (LSD=3.23)	19.8 (LSD=2.80)	

of genes for resistance to BSR and powdery mildew was postulated to explain a positive association between powdery mildew incidence among 'Williams' isolines with different alleles at the *Rmd* locus (resistance to powdery mildew) [21]. This suggestion is confirmed by the findings of this research and that of Polzin *et al.* [25], who mapped the *Rmd* locus to Linkage Group J.

The minor QTL associated with the cluster of RGA3 loci is of special interest because it is the first example of a disease resistance QTL tightly associated with a resistance gene analog. The RGA3 probe was a PCR product made by using primers designed from conserved sequences of known resistance genes from tobacco, flax, and *Arabidopsis* [15]. Thus, it is possible that the gene responsible for the minor BSR resistance QTL is evolutionarily related to other cloned resistance genes.

Introgression of BSR resistance into elite germplasm has been more difficult than desired because of low heritability of resistance and the amount of resources required for the various resistance assays. The marker loci identified in this study to be most closely associated with resistance should be of value to soybean breeders for use in marker-assisted selection. These AFLP and RFLP markers are publicly available. In addition, PCR-based assays have been developed from two of these RFLP markers (data not shown) and are available upon request. Future research involving the genomic sequencing of this region will lead to the development of markers based on resistance gene sequences, rather than on associated markers, to be used in precise introgression of alleles conferring resistance to multiple diseases.

Soybean breeders introgressing resistance from BSR 101 may not need to introgress both the major and minor QTL to achieve acceptable resistance levels. Introgression of the major QTL may be sufficient, since the LSD values indicated no epistasis between the QTL and since the effect of the minor QTL was not significant in the presence of the major QTL in this population (Table 1). Molecular mapping of other brown stem rot resistance genes (Rbs_1 and Rbs_2) would enable tests to determine if their effects would be cumulative and if breeders should try to develop lines with resistance alleles at multiple loci (gene pyramiding).

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